Weak and Partial D phenotypes

A perinatal testing laboratory approach

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Vein to Vein
Calgary
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Conflicts of interest:

Travel funds and honoraria – RedMedEd

Current Methods: CBS perinatal labs in Canada

- Serology
  - AUTOMATED SOLID PHASE (series 4, series 5)
  - MANUAL TUBE (Novaclone)

- Genotyping
  - BIOARRAY BEADchip – IMMUCOR
  - Progenika BLOODchip – Progenika-Grifols

RhD type and Variants

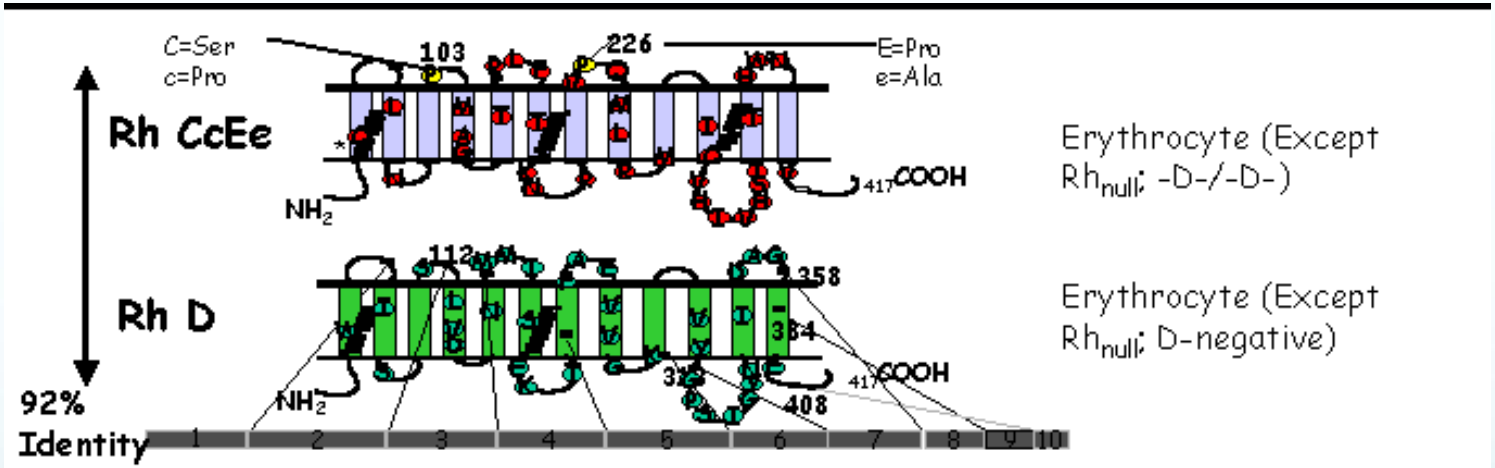
- RH NEGATIVE: 13% (April 2011 – December 2014: 79 103 Rh D Neg/ 608 496 patients)

- VARIANTS: 0.4% of Rh Negative patients (330/79 103)
  - ~65% (213/330) Weak D type 1, 2, 3
  - 117/330 other variants or combinations

RHD and RHCE

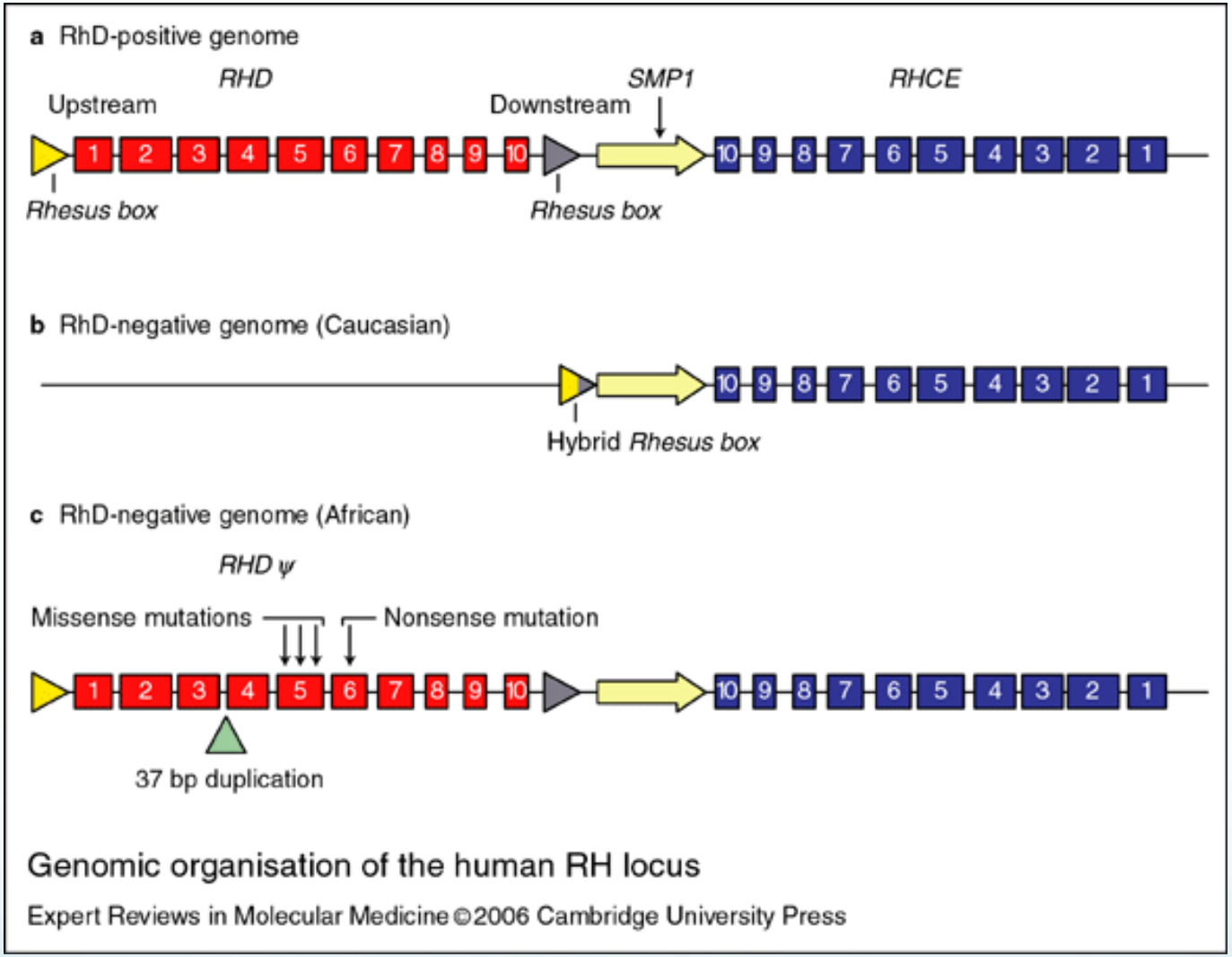
- 2 RH Genes *RHCE* and *RHD*
  - Chromosome 1;
  - 10 exons each
- D and CE vary at only 32 -35 amino acid positions (or are 97% identical)
- C/c and E/e encoded by *RHCE*
- Large transmembrane proteins with multiple epitopes

Rh D and Rh CcEe



How to be D negative….

- Deletion of RHD due to unequal crossover between upstream and downstream genetic elements (15 – 17% in caucasians)
- RHD with 37bp internal duplication and premature stop codon *or* hybrid RHD-CE-D with no D expression (8 - 9% in Africans)
- 10 – 30% of Asians with D negative serology are DEL and *do* express very low levels of D antigen; most arise from deletion or mutation in exon 9



Genomic organisation of the human RH locus

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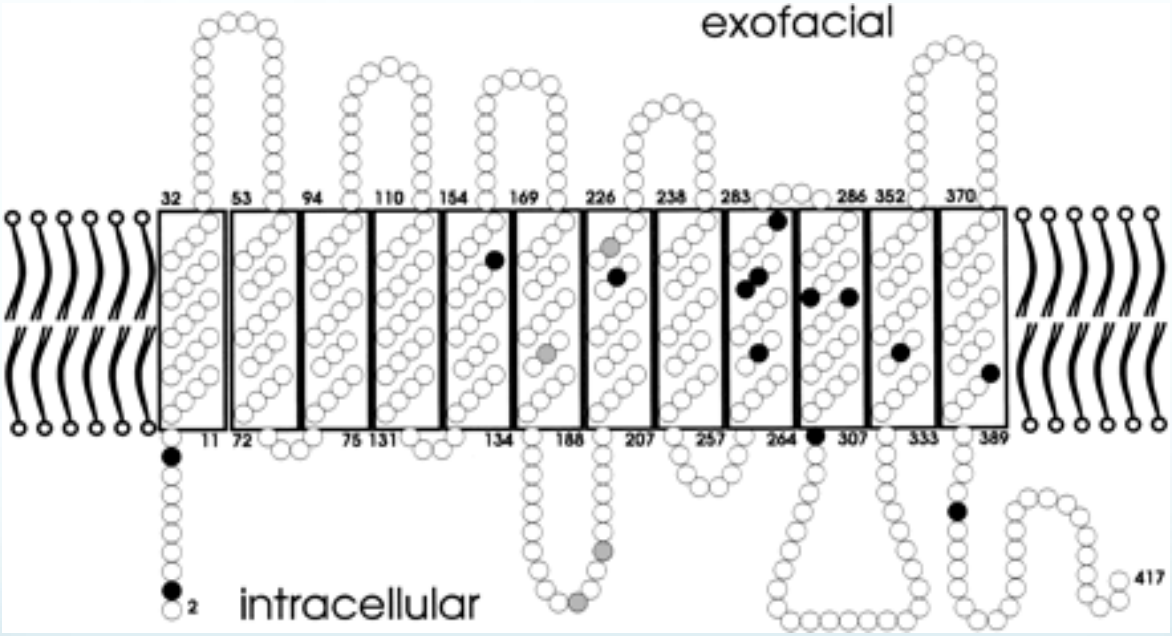
Expert Reviews in Molecular Medicine: http://www.expertreviews.org/
Vol. 8; Issue 13; 12 June 2006

D positive but… Weak and partial D

- Many Many D alleles identified by sequencing studies
- **Weak D** characterized by single or few aa changes primarily in transmembrane or cytoplasmic part of D protein; 0.2 – 1% of Caucasians; may react weakly or not at all in direct agglutination assays
- **Partial D** characterized by aa changes in the extracellular portions of D polypeptide; type as D positive with some antisera

Weak and partial D



Blood, Vol. 93, Issue 1, 385-393, January 1, 1999
Wagner, Gassner, Muller, et al

D epitopes expressed on Rhce proteins

- Some RhCE proteins may have D specific amino acids or a “D-like” region
  - D^{HAR} Partial D antigen with *RH(CE-D-CE)* hybrid gene; and
  - Crawford (ceCF) or *RH(D-CE-D)*
  - May contribute to typing discrepancies with different antisera;
    - very strong reactivity with some antisera, negative with others
  - **Can develop anti D** if transfused with D positive red cells

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Serological variability

- Antigen variables
  - Number of epitopes
  - Presentation of antigen
  - Conformation or accessibility of antigen
- Antibody variables
  - Concentration
  - Immunoglobulin class
  - Avidity or binding constant of antibody
- Method variability

MK Moulds Immunohematology Volume 22; 2, 2006

Commercially available anti D antisera in Canada

- Health Canada approved reagents include:
  - DBL
  - MTS
  - Olympus
  - Alba Bioscience
  - Ortho
  - Gamma
  - Immucor
  - Biotest
- These react with most partial D categories including DII, DIII, DIIIa, DIV, DVa but **NOT DVI**, DVb or FPTT by direct agglutination or gel
- **DVI is detected at IAT phase**

Anti D antisera in common use

- Immucor series 4
  - MS201 IgM
  - MS26 IgG
- Immucor series 5
  - TH28 IgM
  - MS26 IgG
- Novaclone Anti D
  - D175-2 IgM
  - D415 1E4 IgG
- Ortho Tube
  - MAD2 IgM
  - Human IgG
- Gel Card
  - MS201 IgM

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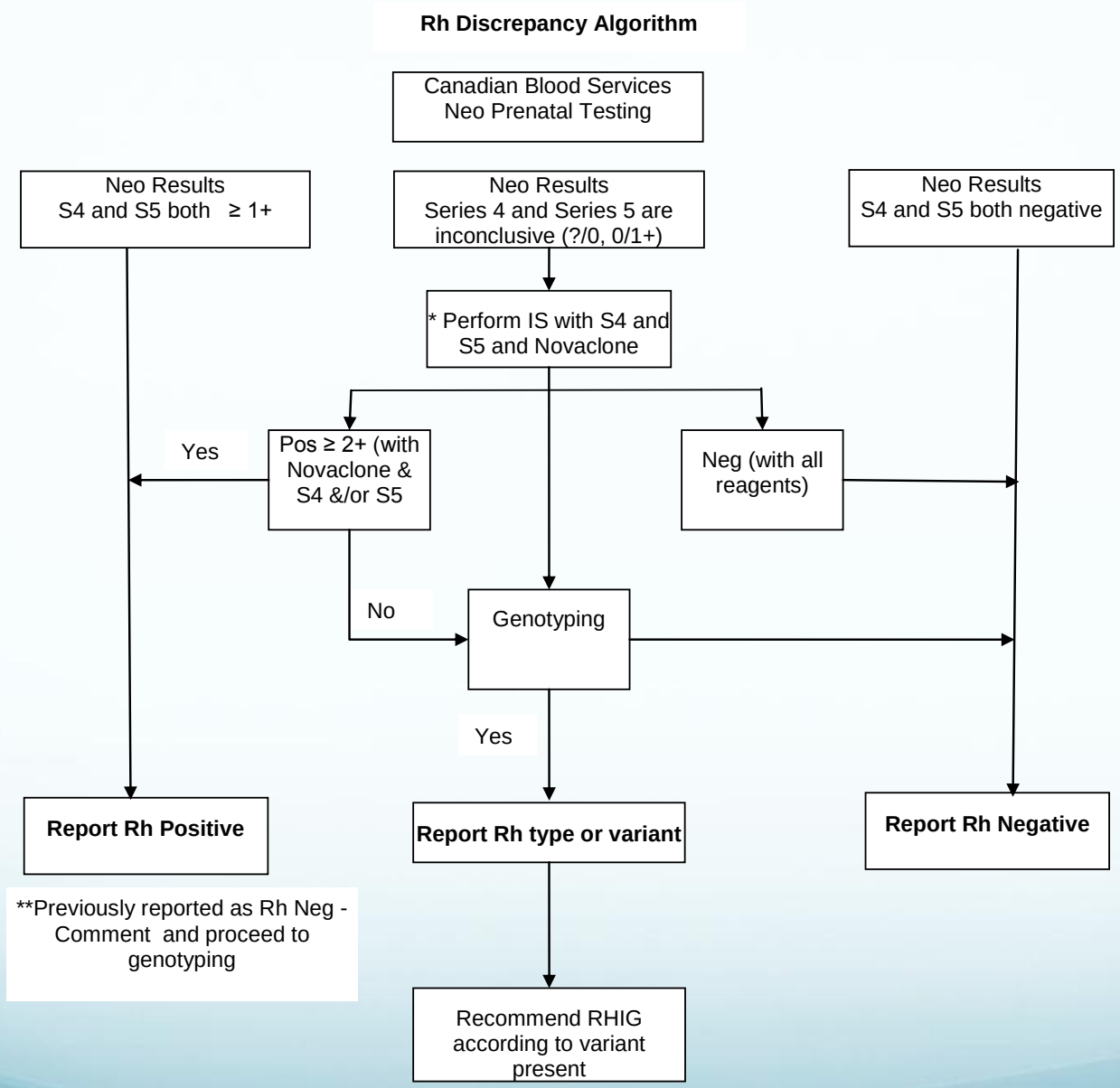
MK Moulds Immunohematology Volume 22; 2, 2006
Immucor Gamma Novaclone Blood Grouping Reagent monograph

Anti D anti Sera

- Monoclonal anti D
  - Antibody directed against a single epitope of the D antigen
  - Produced in vitro from a cell line (recombinant) expressing a particular immunoglobulin gene sequence
  - monoclonals may be “blended”
- Polyclonal anti D
  - A group of anti D antibodies directed against a variety of epitopes on the protein; naturally occurring following an immune response to D immunization.

Reaction strength and phase

- In general, D positive individuals have strongly agglutinated cells in the presence of anti D (3 – 4+)
  - Repeated 1 – 2+ reactivity or ***variability*** between reagents or methods may reflect weak or partial D status
- Weak D testing – or testing at the IAT phase will result in almost all weak and partial D's typing as D positive – including DVI individuals.
- *Avoid IAT testing when D typing except for donors and cord samples*



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Notes on using the serological algorithm…

- Based on comparison of serological and molecular results –allows us to call most weak D 1, 2, 3 positive and capture most other variants
- Serves to limit the number of genotyping studies we perform (and therefore cost)
- We know we miss some partial D variants that react strongly with the anti D anti sera we use (recent DAU cases X 2)
- A (surprising to me) number of variants are not identified by our genotyping (SNP array) method

What do we find?

WEAK AND PARTIAL D VARIANTS

Variant(s)	Total
*RHD*weakDtype1*	119
*RHD*weakDtype2*	45
*RHD*weakDtype3*	49
*RHD*weakDtype3, RHD*weakDtype5*	2
*RHD*weakDtype1, RHD*weakDtype2*	1
*RHD*weakDtype1, RHD*weakDtype3*	3
*RHD*weakDtype1.1*	1
*RHD*weakDtype2, RHD*weakDtype4.0*	1
*RHD*weakDtype4.0*	9
*RHD*weakDtype4.1, RHD*DIIIa-CE(4-7)-D*	1
*RHD*weakDtype4.0, RHD*weakDtype4.3*	6
*RHD*DAR (RHD*weakDtype4.2)*	17
*RHD*DAR, RHD*weakDtype1*	1
*RHD*DAR, RHD*DIIIa-CE(4-7)-D*	1
*RHD*DIIIa-CE(4-7)-D*	1
*RHD*weakDtype5*	1
*RHD*weakDtype9*	1

*RHD*weakDtype21*	2
*RHD*weakDtype42*	5
*RHD*weakDtype42, RHD*IVS8-31C*	1
*RHD*weakDtype55*	1
*RHD*DIVa-2*	1
*RHD*DVII*	14
*RHD*DAUtype3, RHD*807G*	1
*RHD*DAUtype4*	1
*RHD*DAUtype2*	3
*RHD*DAUtype4, RHD*Psi*	1
*RHD*DAUtype5, RHD*Psi*	1
*RHD*DAUtype5, RHD*DIIIa-CE(4-7)-D*	1
*RHD*DFW*	3
*RHD*DOL*	4
*RHD*DLO, RHD*	1
*RHD*DCS*	1
*RHD*DNB*	1
*RHD*DHK*	1
*RHD*1187G* ++new variant	10
*RHD*1060A* ++new variant	1
*RHD*993G* ++new variant	3
*RHD*731T* ++new variant	2
*RHD*IVS1+1T* ++New Exon 1 variant (patient with anti D) RHD	1

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The nature of the variants observed

- Weak D variants very unlikely to form anti D
  - Those few with antibodies – possible autoantibodies ; minimal clinical significance
- Compound heterozygotes with one weak D allele
- Various partial D alleles
- New variants

What’ s next?

Algorithm evolution

- assessment of Novaclone positive/series 4&5 negative by genotyping (BC)
- Established routine genotyping for CBS perinatal testing labs with apparent D variants using BioArray Bead Chip assay
- Based on algorithm 0.4% of patients with at least one serological D negative result met criteria for genotyping
- Of these 61% are weak D 1,2 or 3 and do not require RhIg
- Remainder have undetermined alloimmunization risk and require RhIg

Cell free fetal DNA assessment (cff DNA)

- Small fragments of fetal DNA are found in maternal plasma throughout pregnancy
- Increase in amount with increasing gestational age
- We can determine fetal blood group genotype (along with genotype of many other antigens) by using this DNA from mum's plasma
- Sometimes called NIPT
- Used widely for certain genetic diseases
- Currently used as reference test for women with critical titre antibodies

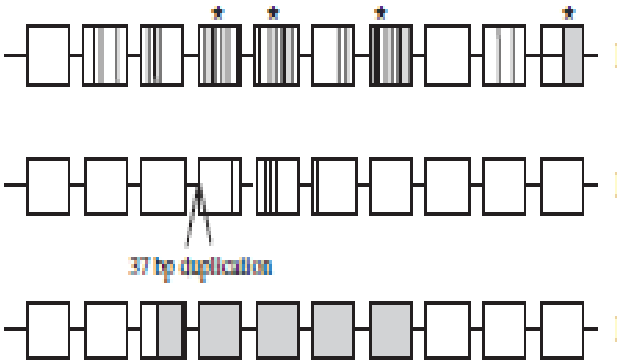

Figure 2 Common D-negative RHD variants. Asterisks in the *RHD* gene denote sites used for PCR amplification by most groups. Bands indicate the RHD specific nucleotides. Bands in the *RHD* pseudogene denote the missense mutations in exons 4 and 5, and the nonsense mutation in exon 6. Shaded exons in *RHD-CE-Ds* represent exons derived from *RHCE* gene.

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Van der Schoot CE, Hahn S, Chitty L. Non-invasive prenatal diagnosis and determination of fetal Rh Status. Seminars in Fetal And Neonatal Medicine (2008) 13, 63-68

… if fetus is antigen negative

- Directed antenatal RhIg Prophylaxis
  - No antenatal RhIg prophylaxis
  - No RhIg for antenatal bleeding events or procedures
  - No cord typing or FMH screening

… if fetus is antigen positive

- No cord testing at delivery (Just give RhIg)
- Follow up care is needed throughout pregnancy for women with critical titre antibodies and an antigen positive fetus

Implication of Variants for CF DNA Testing

- testing mum and dad at the time of fetal DNA assessment will reveal variants
- Knowing typical affected exon or intron may impact interpretation (or assay development)
- Very small proportion of total Rh negative patients
- For uncertain cases provide RhIg to mum

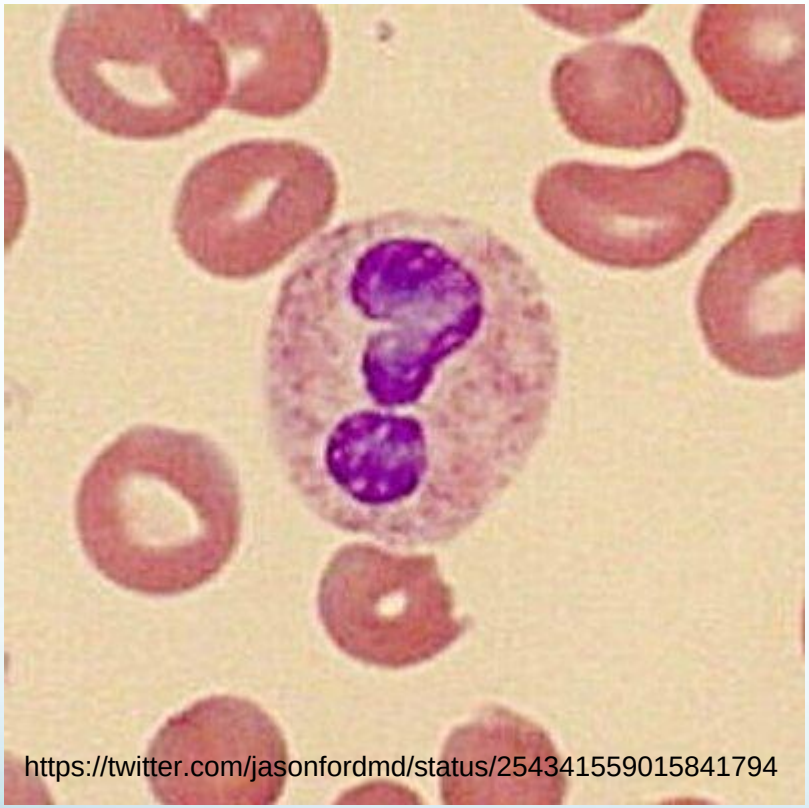
Next steps

- Enhance testing for mums with critical titre antibodies
- Develop local expertise and methodology for CF DNA testing
- Seek funding to develop test and logistics
- Knowledge translation to change our monitoring strategy and keep obstetrical care providers informed

- ***Someday soon*** ?
  - Publication of consensus proceedings
  - Development of a business case
  - Directed antenatal RhIg prophylaxis

Thanks and Questions

Comments & discussion



https://twitter.com/jasonfordmd/status/254341559015841794

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